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Characterization of *Gluconacetobacter diazotrophicus* Isolated from *Sugarcane* (*Saccharum officinarum*) Cultivated in Upper Egypt.

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Abstract

Sugarcane (cv. C 9) cultivated in three governorates of upper Egypt (El-Menia, Assiut, Quena) were sampled to assess the occurrence and characteristic of the endophytic *G. diazotrophicus* isolated from tissues of plants, as well as to determine its abundance in tissues and rhizosphere soil. The 14 endophytic isolates obtained from tissues (roots and stem buds) showed phenotypic and biochemical characteristics identical to those internationally reported in Bergey's Manual of Systemic Bacteriology (2005). No variation were recorded in cell morphology of isolates, or colony characteristic and pigmentation, or physiological traits due to area of plantation or to N - fertilization scheme practiced. The N_2 - fixing activity of the isolated strains ranged from 12.2:17.6 mg N/ g sucrose consumed from culture media (LGI). The enumeration, by the MPN technique on LGI semisolid medium, for the endophytic cells in tissues of sugarcane grown in Assiut Exptl. Farm of faculty of agriculture, recorded mean numbers of 2.0 – 15.4 × 10^5/ g tissues, whereas mean estimated rhizosphere cells ranged from 2.6 – 16 × 10^4/ g of rhizosphere soil.

Keywords: *Endophytes, rhizospheric, diazotrophicus, sugarcane, Gluconacetobacter.*

Introduction

The sugarcane bacteria named *Gluconacetobacter diazotrophicus* that fix atmospheric nitrogen, which was first isolated by Cavalcante and Döbereiner in (1988) from Brazilian sugarcane tissues (endophytic), is mainly characterized by its preference for growth in presence of high sugar concentration in medium (10% or more up to 30%), and acid environment (pH around 5.0 - 5.5), and microaerophilic aeration. Since its first isolation, several reports were published indicating its endophytic nature and isolation from tissues of sugarcane cultivated in different areas around the world. Also, it has been isolated from tissues of unrelated other plants like sweet potato, coffee, tea, banana, pineapple and rice, as well as from rhizosphere soils of rage, coffee and banana (Muthukumarasamy *et al*., 2002).

Fuentes-Ramèrez *et al.,* (1993) reported that the level of N-fertilization of cultivated sugarcane is the key factor for the successful endophytic existence and association of *G.diazotrophicus* with sugarcane plants. They reported that the existence and density of isolation of this endophytic bacteria from Mexican sugarcane tissues were severely limited when mineral N-fertilizers were supplied in excess (275-300 kg ha^{-1}). This was also reported by other researchers (Muthukumarasamy *et al*., 2002).

In Egypt, sugarcane plantation, particularly in Upper Egypt governorates, is heavily fertilized with mineral nitrogen (200 - 400 kg fed^{-1} yr^{-1}). This probably, may affect the endophytic existence of *G. diazotrophicus* in tissues and association with sugarcane plants.

The present work was conducted to assess the occurrence and characteristics of the endophytic *G.diazotrophicus* isolated from tissues of sugarcane cultivated in soils of upper Egypt governorates (El-Menia, Assiut and Quena), and to determine its abundance in tissues of sugarcane as well as in rhizosphere soil.

Materials and Methods

Isolation of *G.diazotrophicus* from Sugarcane (*Saccharum officinarum L.*) tissues:

Samples of young shoots with healthy roots and sprouting stem buds Fig. (1) were taken from 1 - 3 years old sugarcane plants, commercial cultivar C9 cultivated at three Egyptian governorates of Upper Egypt, namely: El- Menia (Mallawy), Assiut (faculty of Agriculture, Assiut Farm) and Quena (El- Matana sugarcane Farm).

The plant samples were carefully washed with tap water and separated into roots, shoots, or sprouting buds which were then surface sterilized. One gram of the surface- sterilized samples (root or buds) was macerated and homogenized in sterile mortar and pistol with 5 % sterile sucrose solution, and the homogenized suspension was then serially diluted in tubes containing 9 ml sterile 5 % sucrose solution. The diluted plant suspensions were then inoculated on tubes of N- free LGI semisolid medium: K_2HPO_4 0.2g; KH_2PO_4 0.6g; $MgSO_2.7H_2O$ 0.2g; $CaCl_2.2H_2O$ 0.02g; $NaMoO_4.2H_2O$ 0.002g; $FeCl_3.6H_2O$ 0.01g; yeast extract 0.02g; 5ml of bromothymol blue (BTB) 5 % solution in 0.2 N KOH; crystalized sucrose 100g; agar 1.8 g/l (adjusted to pH 5.5 - 6.0) for growth and enrichment of the diazotrophic acetobacters in the diluted samples (Cavalcante & Döbereiner., 1988; Li and McRae., 1991). The inoculated tubes were then incubated for 5-7 days at 28 -30^o C. The highest dilution from each sample showing enriched growth, in the form of subsurface yellow pellicle Fig. (2) was serially sub-cultured on tubes of the N-free LGI semi solid medium (for at least 5-7 times, every 3-5 days) for further enrichment and purification. The enriched tubes, showing yellow thick surface pellicle were then streaked on plates of LGI agar medium (18 g/l) and incubated for 5-7 days at 30^o C for obtaining separated colonies.

Fig. 1. Samples showing roots and sprouting buds of sugarcane plant

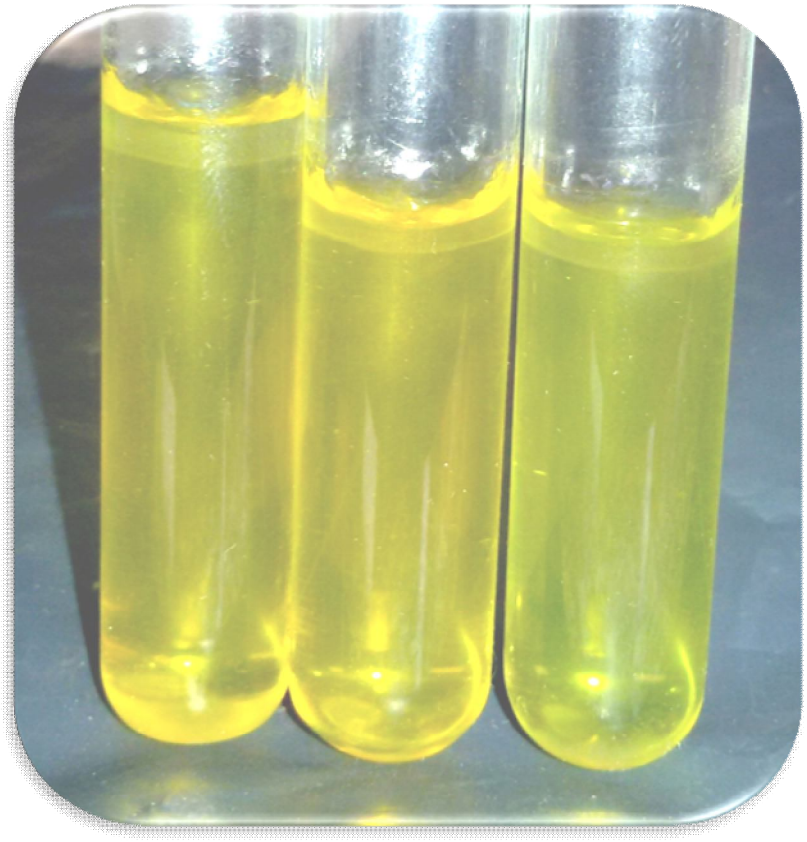


Fig. 2. Three-day's old cultures of isolated strains growing on tubes of LGI semisolid media showing the subsurface yellow pellicle.

Phenotypic properties and biochemical tests performed on strains isolated from sugarcane:

The pure isolates developing on LGI agar medium (3-5 days old) were tested for the following phenotypic and biochemical tests, and were characterized according to Bergey's Manual of Systemic Bacteriology, 2005.

1- Cell morphology: Gram stained smears of the isolates were optically examined, and also determined by scanning electron microscope (Pelczar *et al*., 1957).

2- Cell motility in liquid cultures (48 hr. old) by the hanging drop method, and by stabbing in tubes of LGI medium containing 0.5% agar (Pelczar *et al*., 1957).

3- Catalase reaction by flooding colonies on LGI agar plates with 10% solution of H_2O_2.

4- The ability to utilize various C substrates, (at 0.5 – 1.0 % concentrations), as sole C source in replacement of 10 % sucrose in LGI semi solid medium in tubes.

5- Growth in the presence of 30 % sucrose in LGI semi solid medium in tubes.

6- Growth and colony characteristic and pigmentation on modified 1% ethanol and $CaCO_3$ agar medium in plates.

7- Colony characteristics and pigmentation on modified glucose, yeast extract, $CaCO_3$ (GYC) agar medium in plates.

8- Colony characteristic and pigmentation on potato sucrose agar medium (PS) in plates.

9- Nitrate reduction in liquid LGI medium containing 10 mM of KNO_3.

10- Phosphate solubilization on modified phosphate LGI agar medium.

11- Starch hydrolysis by cultures grown on LGI agar (medium containing 0.5 % soluble starch).

Testing the isolates for N_2-fixing activity:

The ability of the isolates *G.diazotrophicus* strains to fix N_2 in culture medium was tested by two methods:

1- By determining the total nitrogen content of 25 ml of the culture grown for 7 days in liquid LGI medium by the micro-Kjeldahl method (Jackson., 1958), and determining sucrose content in medium and grown cultures colorimetrically by the method described by Dubois *et al.,* (1956).

2- By the acetylene reduction assay for the cultures grown on 10 ml semisolid LGI medium in 30 ml vials that were sealed tightly with rubber stopper and Para film and injected with 10 % acetylene. After one-hr. from injection, 0.25 ml gas samples from the vials were removed by microneedle and injected in the gas chromatography apparatus at the Agricultural Research Center, Giza, Egypt: Hewlett-Packard 5890 fitted with two flame ionization and electron capture detectors and Al_2O_3 capillary column.

Enumeration of *G.diazotrophicus* in rhizosphere soil and in tissues of sugarcane grown in Assiut Exptl. farm:

- **Abundance in rhizosphere soil:**

Young plants of sugarcane cultivar (C9, second ratoon) were uprooted, gently shaken to remove loosely attached soil particles.

Healthy roots were then separated and shaken with gentle agitation in 90ml sterile tap water in 250 ml Erlenmeyer flasks to remove rhizosphere soil adhering to roots surfaces. The rhizosphere soil slurry obtained was serially diluted in tubes each containing 9 ml of sterile 5% sucrose solution. From each dilution, aliquots of 1 ml were inoculated on five tubes of LGI semi solid medium for growth and enrichment of the diazotrophic acetobacter in rhizosphere soil. After 10 days incubation at 30^{o} C, the number of positive tubes in each dilution, showing the typical yellow to orange subsurface pellicle, were used for calculating the MPN of *G.diazotrophicus* / gram of rhizosphere soil using McCradye table (Alexander, 1982).

- **Abundance of endophytic *G.diazotrophicus* in roots, stem buds of sugarcane:**

Samples (1-2g), of the separated washed surface sterilized roots or sprouting buds of sugarcane were macerated and homogenized in 10 ml aliquots of 5 % sterile sucrose solution, then serially diluted in tubes containing 9 ml of sterile 5 % sucrose solution. Aliquots of 1 ml from each dilution were inoculated on 5 tubes of LGI semisolid medium for MPN enumeration of endophytic *G.diazotrophicus* bacteria per gram of fresh plant sample as previously described in rhizosphere soil (Alexander, 1982). Tubes showing yellow to orange subsurface pellicle after 10 days incubation were considered positive.

Results and Discussion

Fourteen pure isolated strains were finally obtained after repeating subculturing of macerated sugarcane tissues and streaking on plates of LGI agar medium. Table (1) shows labels and source of isolated strains, plantation area, soil characteristics and N- fertilization scheme followed.

The identification of the isolated bacterial strains from tissues of sugarcane and confirmation of their belonging to the species *Gluconacetobacter diazotrophicus* were based on colony characteristics of the isolates on the specific agar media as well as on diagnostic phenotypic and biochemical tests as reported by Cavalcante & Döbereiner (1988) and described in Bergey's Manual of Systemic Bacteriology (2005).

Table 1. Labels and sources of endophytic *G .diazotrophicus* strains isolated from *sugarcane* (cv.C9) and plantation area of host plant.

plant	Plantation area of host plant	Soil texture and pH(1:2.5) suspension	Scheme of N –fertilization of host plant (kg N / fed)	Plant stage at sampling	Label of isolated strains	Source of isolates
Sugarcane	Quena Matana Ex-perimental station	Clay pH 7,5	210	First ratoon	M_{b1}	Young stem buds
					M_{b2}	Young stem buds
					M_{b4}	Young stem buds
					M_{r5}	Roots
					M_{r7}	Roots
					M_{r8}	Roots
	El-Menia Mallawy Experimental station	Clay pH 8,0	186	Third ratoon	El-M_{r1}	Roots
					El-M_{r2}	Roots
					El-M_{b1}	Young stem buds
					El-M_{b2}	Young stem buds
	Assiut Farm of Fact. of Agriculture	Clay pH 8,1	186	Second ratoon	$As_1 r_1$	Roots
					$As_1 b_2$	Young stem buds
				Eighth ratoon	$As_2 r_1$	Roots
					$As_2 b_2$	Young stem buds

Colony characteristics of the isolated strains developing on LGI agar medium:

Colonies of the isolated strains from sugarcane tissues (roots or stem buds) on LGI agar plates (14-days old) were small, 2-3 mm in diameter, gummy, smooth, round, yellow to deep orange of entire margin. Fig. (3) shows size, pigmentation and colony characteristics of some selected strains isolated from sugarcane tissues. No differences in pigmentation or colony characteristic were detected due to source of isolation (roots or stem buds) or due to sugarcane plantation area (Assiut, El-Menia or Matana).

Colony characteristics of the isolated strains developing on PS agar plates:

All strains isolated from sugarcane produced large slimy brown colonies, 3- 4 mm in diameter, Fig. (4).

Colony characteristics on ethanol-$CaCO_3$ agar medium:

The isolated strains from sugarcane (roots or stem buds) produced distinguishable large blue-green gummy colonies with deep blue centers, probably due to precipitation of dissolved $CaCO_3$. Fig. (5).

Colony characteristics on glucose-$CaCO_3$ agar medium (GYC):

Fig. (6) shows the characteristics colonies produced by some selected isolated strains on plates of GYC agar medium. The growth pattern of colonies produced on this medium were similar to those produced on ethanol- $CaCO_3$ agar medium (blue green gummy). But after 3-weeks of incubation, the colonies and the medium in the plates turned to dark brown, probably due to over oxidation of the glucose present in the medium.

M_4 $As_1\ r_1$ El-M_{r1}

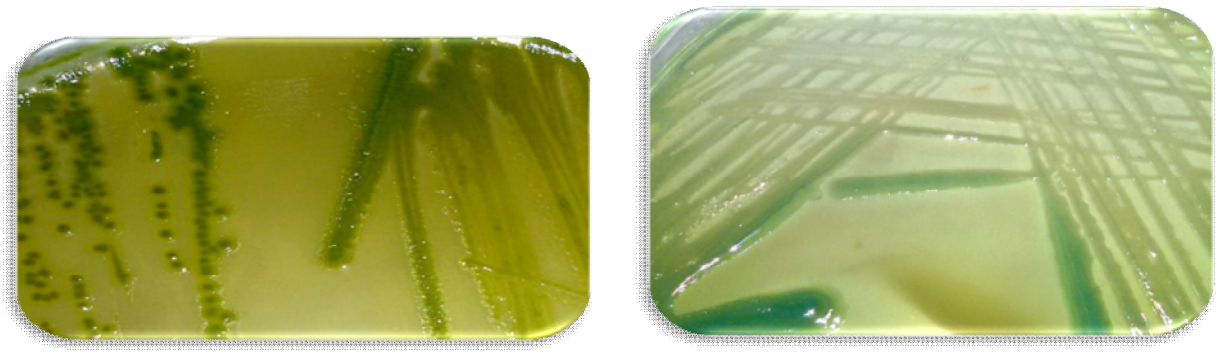
Fig. 3. Colonies, 10-14 days old cultures, of some of selected isolated endophytic *G.diazotrophicus* strains on LGI agar medium: (M_4) strain from Matana sugarcane; ($As_1\ r_1$) strain from Assiut sugarcane; (El-M_{r1}) strain from EL-Menia sugarcane.

M_1 $As_{1\ r1}$ EL-$M_{\ b1}$

Fig. 4. Colonies (2 weeks old culture) of some selected isolated endophytic *G.diazotrophicus* strains on PS agar medium: (M_1) strain from Matana sugarcane; ($As_{\ r1}$) strain from Assiut sugarcane; (EL-$M_{\ b1}$) strain from EL- Menia sugarcane.

$As_2\ r_1$

Fig. 5. Colonies (2 weeks old cultures) of some selected isolated endophytic *G.diazotrophicus* strains on ethanol agar medium; ($As_{\ 2}\ r_1$) strains from Assiut sugarcane.



M_2 $As_2\ r_1$

Fig. 6. Colonies (2weeks old culture) of some selected isolated endophytic *G.diazotrophicus* strains on glucose agar medium; (M_2) strain from Matana sugarcane; ($As_2\ r_1$) strain From Assiut sugarcane.

Diagnostic phenotypic and biochemical tests performed for identification of the isolated strains:

Table (2) shows the results obtained for the tests performed on the isolated strains.

Cell morphology:

Optical examination of gram stained smears from the agar colonies of isolated strains on LGI plates (5 - 7 days old culture) showed non-spore former unicellular G (-) short rods, 1 - 1.5 µ in length and 0.5 µ in width. Fig. (**7**) present scanning electron microscope photo (10000 magnification) of cells of the isolated strain As_1 r_1 grown on Ps medium. The photo shows the same cell description obtained from optical examination: unicellular rods 1 - 1.25 µ in length x 0.5 - 0.75 µ in width, with round ends and non-spore formers.

Cell motility:

The liquid cultures (3-5 days old) of all isolated strains gave negative results for motility test by the hanging drop technique. But, when motility of the strains was tested by stabbing small part of the agar colonies with straight needle in tubes of LGI semi-solid medium (containing 5 g agar/l), all isolated strains gave positive motility results. Fig. (8) shows the growth pattern of three of the isolated strains in tubes of the semi-solid medium after 10 days of incubation.

The positive motility results obtained by the stabbing technique is probably due to the suitable microaerophilic conditions and better growth of the isolated strains in the semi-solid medium in comparison to their slow growth in the liquid medium.

Table 2. Results of phenotypic and biochemical tests performed on isolated strains of *G.diazotrophicus*

Tests	Sugarcane isolates from Assiut, El-Menia and Matana (14 strains)
Cell morphology by optical examination	Single G(-), non-spore former rods
Motility tested by stabbing technique	+
Growth in tubes of LGI semi solid medium	Sub surface yellow to orange pellicle and acid production
Growth in presence of 1% ethanol	++
Growth in presence of 30% sucrose	++
Catalase reaction	++
Nitrate reduction	-(13)* +(1)*
Phosphate solubilization	++
Starch hydrolysis	-
N_2-fixing activity	+ (from 12.2- 17.6 mg N/ g sucrose)

* Number of strains.

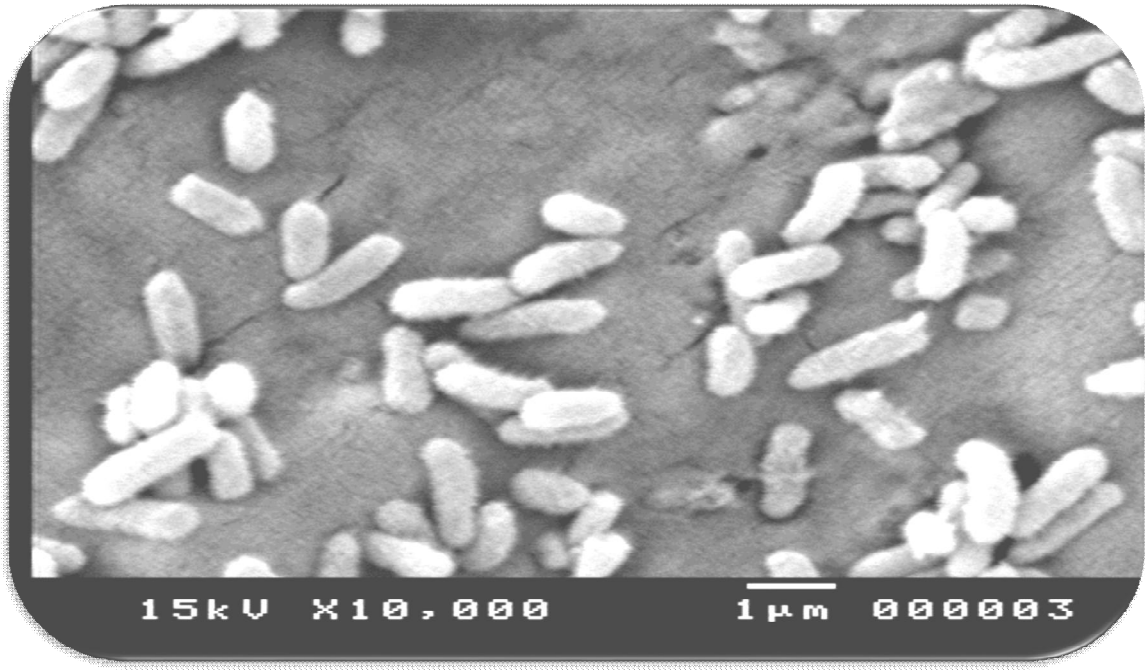


Fig. **7**. A scanning electron microscope picture for cells of isolated strain $As_{l\ rl}$ from Assiut sugarcane root (10000 x magnification) for *G .diazotrophicus* growth on PS medium.

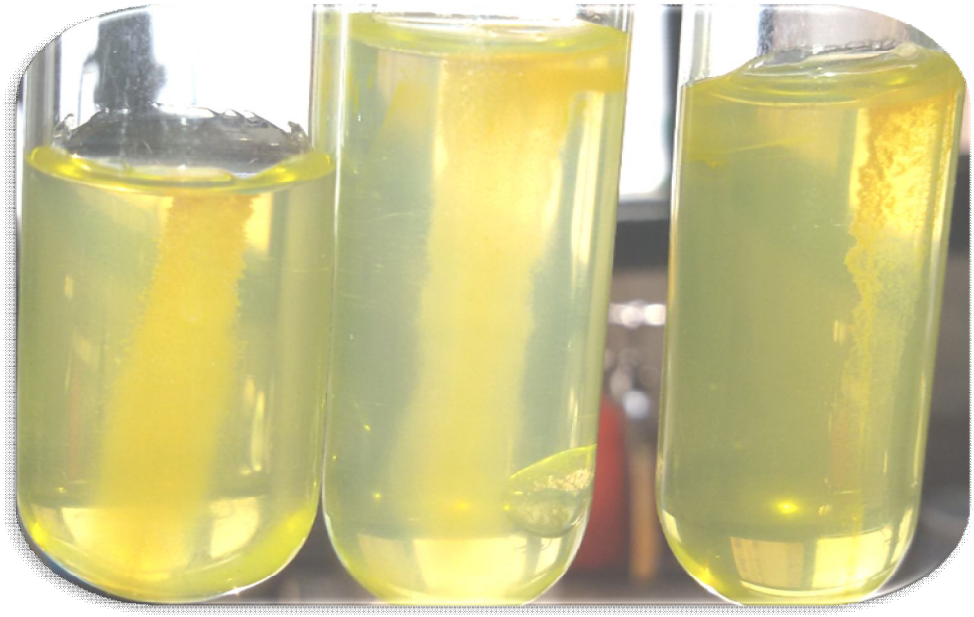


Fig. 8. Three tubes showing motility of isolated strains inoculated by stabbing in tubes of LGI medium containing 0.5% agar.

Other biochemical tests performed on isolated strains:

Table (2) shows that all isolated strains from sugarcane gave positive results for catalase reaction, growth in presence of 30% sucrose or 1% ethanol and positive phosphate solubilization. But gave negative results for starch hydrolysis and nitrate reduction tests. The good growth obtained with 30% sucrose in the medium is a distinguishable character indicating the ability of this species to grow in presence of high sugar concentration.

Ability to use different sugars and certain organic materials as sole C-source:

Results in Table (3) indicate that most of the isolated strains from sugarcane were able to use glucose, fructose, rhamnose, arabinose, lactose, maltose, mannitol and citrate as sole carbon source for growth in LGI medium with acid production in most cases. But K-malate was the only tested organic substance that was not assimilated and used for growth by all isolated strains.

N_2-fixing activity of the isolated stains:

The nitrogenase activity of the strains determined by the AR assay showed great variations, recording from 0.04 to 470 n mole ethylene/ml/hr. the highest activity was recorded for EL-Menia strain (El-Mb_1) isolated from buds, and the lowest were recorded for the strains isolated from El-Matana sugarcane buds (0.04 - 0.8 n mole/ml/hr. The reason for this great variation in activity of the enzyme determined by this technique is not known, but may be due to difference in rate of growth of the isolated strains and consequently in the enzyme capacity to reduce acetylene. On the other hand, the capacity of N_2-fixation by the isolated strains grown for 7-days on LGI medium and determined by the micro-Kjeldahl technique, gave close results, recording between 12.2 - 17.6 mg N fixed/g sucrose consumed. These results mean moderate N_2-fixing capacity of the isolated strains.

Table 3. Numbers of isolated strains growing on different sugars and C- sources, in replacement of sucrose (10%), in LGI semisolid medium

Tested C- sources and concentrations	Sugarcane isolates from Assiut, El-Menia and Matana (14 strains)
Glucose 0.5%	14 (+)
Fructose 0.5%	14 (+)
Rhamnose 0.5%	14 (+)
Arabinose 0.5%	12 (+) 2 (-)
Lactose 0.5%	14 (+)
Sucrose 1%	14 (+)
Maltose 1%	12 (+) 2 (-)
Mannitol 1%	12 (+) 2(-)
Ethanol 1%	14 (+)
k.malate 0.5%	14 (-)
sodium citrate	12(+) with no acid production 2 (-)

Abundance of *G.diazotrophicus* in rhizosphere soil and in tissues of sugarcane (cv. C9) grown at Assiut Exptl. Farm:

Table (4) shows numbers of *G.diazotrophicus* in rhizosphere soil and tissues of sugarcane (endophytic) determined by the MPN technique (Alexander, 1982) in two trial determinations and using LGI semi-solid medium for cultivation.

Mean numbers estimated for endophytic cells in tissues (roots and stem buds) ranged from 2.0 – 15.4 × 10^5/g, whereas the numbers in rhizosphere soil (associative) were lower than those in tissues and ranged from 2.6 - 16 x 10^4 /g soil. These recorded numbers indicate the establishment of this bacterial species in both soil and tissues of sugarcane cultivated in Assiut Exptl soil in spite of its alkaline pH(8.0 - 8.1) and heavy N-fertilization scheme (200 kg N/ fed.yr^{-1}) and dry weather of the area.

The high enumeration results obtained in this investigation for endophytic *G.diazotrophicus* in sugarcane are in contrast to the reports of Fuentes-Ramèrez *et al*., (1993) and Muthukumarasamy., (1995), indicating that supplementation of N- fertilizers in excess severely limit the endophytic existence of this species in sugarcane tissues.

The high rhizosphere numbers of G.diazotrophicus recorded in the present investigation are in accordance with those reported by other investigators (Li and McRae., 1991 and 1992), and are probably due to secretion of soluble sugars in root exudates and provision of C- sources and favorable environment (acidity and partial aeration) for flourishing of this species in rhizosphere of sugarcane.

Table 4. Most Propable No. of *G.diazotrophicus* in rhizosphere soil and plant parts of sugarcane growing in Assiut Experimental Farm

Sugarcane	**First determination* (g^{-1})**	**Second determination* (g^{-1})**	**Mean of the two determinations (g^{-1})**
Rhizosphere soil	2.6×10^5	16.0×10^4	21×10^4
Plant- Roots	2.8×10^6	2.8×10^5	15.4×10^5
stem Buds	1.7×10^4	4.0×10^5	2.1×10^5

*Samples were taken on 15/11/2015 and 30/11/2015 in the first and second determinations, vespectively.

Reference

Alexander, M. 1982. Most Probable Number Method for Microbial Populations. *In* A.L. Page *et al*. (ed.) Methods of Soil Analysis. Part 2. 2nd ed. Agronomy 9:815-820.

Bergey's Manual of Systematic Bacteriology. 2005. 2nd ed . Vol ∏. The proteobacteria, 72-77.

Cavalcante, V. A. and J. Döbereiner. 1988. A new acid-tolerant nitrogen-fixing bacterium associated with sugarcane. Plant and Soil, 108: 23- 31.

Dubois, M.; K.A.Gilles; J.K. Hamilton; P.A. Rebers and Fred Smith.1956.

Colorimetric Method for Determination of Sugars and Related Substances. Analytical Chemistry, 28(3): 350- 356.

Fuentes-Ramèrez, L. E.; T. Jime ènez-Salgado; I. R. Abarca-Ocampo and J. Caballero-Mellado. 1993. *Acetobacter diazotrophicus*, an indoleacetic acid producing bacterium isolated from sugarcane cultivars of Meèxico. Plant and Soil, 154: 145- 150.

Jackson, M. L. 1958. Soil chemical analysis. Prentice-Hall, Inc. Englewood Cliffs, New Jensy, pp: 498.

Li, R. and I. C. McRae. 1991. Specific association of *diazotrophic Acetobacters* with sugarcane. Soil Biol. Biochem., 23: 999– 1002.

Li, R. and I. C. McRae. 1992. Specific identification and enumeration of *Acetobacter diazotrophicus*, in sugar cane. Soil Biol. Biochem., 24: 413– 419.

Muthukumarasamy, R. 1995. Endophytic nitrogen-fixing bacteria from sugarcane in India. In: Proceedings of the International Symposium on Sustainable Agriculture for the Tropics: the Role of Biological Nitrogen Fixation, Rio de Janeiro, Brazil. pp: 216-217.

Muthukumarasamy, R.; G. Revathi; S. Seshadri and C. Lakshminarasimhan. 2002. *Gluconacetobacter diazotrophicus* (syn. *Acetobacter diazotrophicus*), a promising diazotrophic endophyte in tropics. Curr. Sci., 83: 137 -145.

Pelczar, M. J., Jr., *et al*…, 1957. Manual of microbiological methods Society of American Bacteriologists. McGrow-Hill, Book Company, New York.

تعريف بكتيريا *Gluconacetobacter diazotrophicus* المعزولة من قصب السكر المزروع فى مصر العليا

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قسم الأراضى والمياه– كلية الزراعة– جامعة أسيوط

الملخص

أخذت عينات من نبات قصب السكر صنف C9 من ثلاث محافظات بصعيد مصر (المنيا – أسيوط – قنا) لاختبار وجود ووصف بكتيريا *Gluconacetobacter diazotrophicus* ، وكذلك تقدير سيادة هذه البكتيريا فى أنسجة النبات (جذور، سيقان، براعم، تربة منطقة الريزوسفير)، نتج عن عملية العزل للبكتيريا ١٤ عزلة، وبدراسة الصفات الظاهرية والصفات البيوكيمائية للبكتريا المعزولة كانت مشابهة لتلك التى جاءت فى المرجع الدولي للبكتيريا Bergey's Manual of Systemic Bacteriology (2005). كما أنها لم تسجل اختلافات فى الشكل الخارجي للخلايا أو فى صفات المستعمرات أو افراز الصبغات أو الصفات الفسيولوجية بسبب أختلاف مناطق أخذ العينات أو نظام تسميد النيتروجين وتراوحت كفاءة تثبيت النيتروجين ما بين ١٢,٢ : ١٧,٦ ملجم نتيروجين لكل جرام سكروز مستهلكة فى بيئة LGI، أيضاً تراوحت أعداد البكتيريا المعزولة من القصب النامي فى مزرعة كلية الزراعة بأسيوط ما بين ٢,٠ :١٥,٤ ×١٠ ^{٥}/ جم نسيج القصب و ٢,٦ : ١٦ × ١٠ ^{٤}/جم تربة الريزوسفير.

الكلمات الدالة: *قصب السكر– تثبيت النيتروجين – الريزوسفير .*